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SEPARATION OF HIGHLY-ACTIVE ALLERGEN FROM THE GAISKI TULAREMIA STRAIN NO. 15

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Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yё or ё. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

SEPARATION OF HIGHLY-ACTIVE ALLERGEN FROM THE GAYSKIY TULAREMIA STRAIN NO. 15

S. I. Chernyakhover, L. V. Sirotyuk, and
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For the purpose of clearing up the immunity to tularemia extensive use is made of the cutaneous test with tularin, which is a heated suspension of tularemia microbes. The use of a suspension of killed bacteria for the diagnosis of tularemia in humans was proposed by Bychkov and Rappoport (1931) and Foshay (1932).

Mayskiy and Shipitsina (1951, 1952), using the method of extraction with trichloroacetic acid, prepared from a virulent tularemia strain a soluble preparation which they called tuallergen. The intracutaneous administration of the preparation in a dose of 100-10 μg caused a specific reaction already in 5-10 min in vaccinated persons and in those who had had the disease. The authors proposed to use tuallergen for the accelerated diagnosis of tularemia. Subsequently Shipitsina et al. (1961) prepared tuallergen-2 from the vaccine strain. Tolstukhina and Ivanov (1953), who processed the vaccine strain by using Buaven's method, obtained a preparation which when administered in a dose of 100 μg into the skin of guinea pigs caused a reaction which was similar to the reaction to the administration of tularin.

Vareninova et al. (1956), by means of extraction with acetic acid, isolated from tularemia bacteria a polysaccharide fraction which possessed a high degree of specificity and in the case of intracutaneous administration caused a reaction in a dilution of 1:100,000.

This preparation was tested in a dilution of 1:100 on humans by setting up the allergic test by the cutaneous method (Kontorina, 1958).

The number of works on the obtaining of "chemical" allergens and on the study of the role of the individual cell components is not very great (Shipitsina, 1956; Shipitsina et al., 1961). In connection with this we undertook the task of obtaining fractions of the tularemia microbe by various methods and studying their allergenic properties and chemical composition.

A culture of restored standard Gayskiy No. 15 vaccine strain was incubated on solid fish or placental agar medium with the addition of cystine and glucose for 48 hours, then washed with an isotonic solution of sodium chloride at pH = 7.0 and dried with acetone. The fractions were separated from the dry bacteria by means of their processing with trichloroacetic acid in the Ruaven method, acetic acid in the White method, and tryptic digestion using the method of Topli and Raystrik. Extraction was carried out using the method of Westfall at 65°, and also ether treatment in the Larsen method. Chloroform treatment was carried out by means of the addition of 40% (by volume) chloroform to a 2% aqueous suspension of microbes. The suspension was held at room temperature for 48 hours and periodically agitated.

All the preparations were centrifuged at 4,500 rpm for two hours and dialyzed against tap and distilled water with the subsequent lyophilic drying.

In the preparations obtained the chemical indices were determined - total nitrogen, protein, reducing substances, total phosphorus, and nucleic acids.

Total nitrogen was determined by colorimetric analysis with Nessler reagent, reducing substances - according to Hagedorn-Jensen, protein - by Lowry's method, total phosphorus - by the method of Fiske and Suborrow, and the content of nucleic acids - by the spectrophotometric method in the Spirin varient.

Table 1 shows the chemical characteristics and yield of preparations obtained by the various methods. The indices given are average for three-four series.

Table 1. Chemical characteristics of preparations obtained from tularemia microbes by various methods.

Method of obtaining	Yield of preparation (in % to weight of dry bacteria)	Chemical composition of preparations (in % for dry substance)				
		Total nitrogen	Reducing substances	Total phosphorus	Nucleic acids	Protein
Extraction by Chloroform	18.15	10.9	22	2.8	31.7	17.2
Extraction by Ether	20	10.2	19.6	3.5	28	17.3
Extraction by Trichloroacetic acid	2	4.8	37	0.4	1.2	5
Extraction by Acetic acid	7.5	8.4	34.4	0.2	1.3	8.6
Tryptic digestion	4.1	5.4	28.6	0.3	0.6	14.1
Treatment with phenol	16	9.8	29.6	3.4	16	3.2

The capacity of the preparations obtained to cause a cutaneous allergic reaction was checked on guinea pigs 4 weeks after their immunization with 20 million bacteria of the vaccine strain. The preparation in doses of 0.1 ml was introduced strictly intracutaneously. As a control the same guinea pig was given tularin in

the same volume intracutaneously. The reaction was considered in 24 hours.

The results of the titration of the preparations for the purpose of establishing the dose which causes a reaction in guinea pigs similar to the reaction to tularin showed that the greatest allergenic activity was possessed by preparations which were treated with chloroform and ether. The least activity belonged to the preparation which was obtained following treatment with phenol at 65° (dose of 20 µg caused a reaction which was less expressed than the reaction to tularin).

Further investigations were conducted with the preparation obtained by the method of chloroform extraction, since this method was the least complex, gave the greatest yield of preparation, and the allergen obtained turned out to be the most active.

For clarifying the completeness of the extraction of allergen a five-fold repeated extraction was carried out. It turned out that part of the allergen (around 70-80%) was extracted already during the first treatment. The further extraction of preparation was complicated by the formation of a suspension which was difficult to separate.

In order to clear up the time of the onset of the specific reaction the chloroform allergen, tularin, and allergens obtained by various methods from anthrax and plague bacteria, and also tuberculin and gamma-globulin were administered intracutaneously to guinea pigs which had been sensitized by the administration of 20 million bacteria of the vaccine strain. In all the pigs in those cases when non-specific preparations were administered in 20 min edema was noted at the site of administration of the preparation. It was lessened by 3 hours and disappeared by 24 hours. Only following the administration of chloroform allergen and tularin was the reaction sharply expressed after 24 hours. By 48 hours the reaction to both preparations was gradually reduced.

In connection with this we deemed it possible to consider the reaction after the administration of chloroform allergen after 24 hours.

For studying the sensitizing properties the chloroform allergen was administered to guinea pigs 12 times with intervals of from 2 to 7 days for a period of 1.5 months. The dose of allergen for each administration was 1 μg. The tests showed the absence of a sensitizing action for allergen with this particular schedule at the same time that tularin already in the case of repeated administration conditioned a cutaneous allergic reaction.

The possibilities of sterilization of the preparation by flowing steam by the conventional method, autoclaving for 30 min at 110° and 20 min at 120°, were studied. Prior to sterilization the preparation was diluted with an isotonic solution of sodium chloride or a phosphate buffer with 3% glycerin to a content of one dose in 0.1 ml. As a result of these tests we accepted a 30-minute sterilization at 110° of a preparation which was diluted in a phosphate buffer with 3% glycerin. Since during the process of sterilization a certain lowering of the activity of the preparation was observed, the concentration of allergen was increased to 2 μg in a dose. A preparation in such a concentration caused a reaction which was similar to that to tularin (checked on 85 guinea pigs).

For the purpose of studying the nature of the allergenic onset the preparation was subjected to fractionation with ammonium sulfate. Fractionation was carried out on three series of chloroform allergen obtained from various batches of bacteria mass. Fraction I was a preparation which was obtained at 50% saturation, the second fraction - at 100% saturation, and the third fraction - the part which remained after removal of fractions I and II.

After dialysis and lyophilic drying the allergenic activity of the fraction was checked and the chemical composition determined.

As can be seen from Table 2, the first fraction was active, and the third, which consisted mainly of nucleic acids, did not possess allergenic activity. The second fraction was characterized by a various degree of activity, which possibly depended on the content of protein in the given preparation (fraction II of series No. 4 contained 1.0% protein and caused a cutaneous reaction with a diameter of 0.4 cm, fraction II of series No. 6 with a 13.2% content of protein - a cutaneous reaction with a diameter of 1.2 cm).

The amount of reducing substances in all the preparations fluctuated within the limits of 10.8-22.6% with a tendency for a decrease in the content of polysaccharide in fractions as a measure of their dilution.

Table 2. Characteristics of the fractions of chloroform allergen.

No. of series	Fraction	Yield of preparation (in %)	Chemical composition of fraction (in %)			Average size of reacting sector of skin (in cm)	
			Protein	Reducing substances	Nucleic acids	Investigated fraction	Control (tularin)
4	I	40.4	13.6	21.4	41.5	1.1	1.4
	II	7.25	1	17	22.3	0.4	1.9
	III	12.2	2	15	86	—	1.7
6	I	35.7	14.7	22.8	37.2	1.[illegible]	1.6
	II	2.8	13.2	16.2	42.3	1.2	1.7
	III	2.6	0.9	12	91.1	—	1.9
7	I	34.8	15.3	22.2	39.4	1	1.6
	II	4.5	7.5	10.8	25.2	1.3	1.4
	III	17.8	0.5	12	84.5	—	1.4

Conclusions

1. By making use of various methods a number of allergen preparations were obtained from a tularemia microbial mass (vaccine strain). Studies were made of the chemical composition and activity (based on the skin test).

2. The most expressed capacity to cause a cutaneous reaction in sensitized animals was possessed by the preparation obtained by the chloroform method. In a dose of 0.5-1 μg it caused the same reaction as tularin.

3. Following the fractionation of the chloroform allergen by the method of salting-out with ammonium sulfate three fractions were obtained. Of these I and II were active, and III, consisting mainly of nucleic acids, did not possess the properties of the allergen.

4. The conditions of sterilization of the preparation were studied. Sterilization is recommended in a phosphate-saline mixture with 3% glycerin at 110° for 30 min (concentration of allergen 2 μg in a dose).

Bibliography

Бычков В. А. и Раппопорт Л. Г. ВМЭП, 1931, т. 10, п. 4, 327.

Варенцова Н. К., Конторина А. А. и Бахрах Е. Э. ЖМЭИ, 1956, 2, 8.

Конторина А. А. Природноочаговые заболевания. Сб. тр. межинст. конф., посвящен. 50-летию Томского НИИВС, 1958, 306.

Майский И. Н. и Шипицина Г. К. Бюлл. экспер. биол. и мед., 1951, 3, 66.

Майский И. Н. и Шипицина Г. К. Бюлл. экспер. биол. и мед., 1952, 2, 60.

Толстухина Е. Н. и Иванов В. И. Бюлл. по обмену опытом, 1953, 1 (42), 50.

Шипицина Г. К. Химический состав и биологические свойства антигенного комплекса возбудителя туляремии. Автореф. дисс. М., 1956.

Шипицина Г. К., Самельсон Р. А., Родионова Л. В. и Колядицкая Л. С. Бюлл. экспер. биол. и мед., 1961, 9, 83.

Foshay L., Journ. Inf. diseases, 1932, v. 51, N 2, 286.